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PHYLOGENETIC ANALYSIS OF THE SWINE LEUKOCYTE ANTIGEN-6 GENE GENERATED FROM SPLEENTISSUE OF KOREAN NATIVE PIG

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ABSTRACT

This study was aimed to search genetic variants, to investigate phylogenetic relationships between pig breeds and to provide basic genetic information of Korean Native Pigs (KNP) using the variations of the Swine Leukocyte Antigen-6 (SLA-6) gene. Cloning of the Swine Leukocyte Antigen (SLA)-6 gene in the MHC non-classical region was performed with spleen tissues of Korean Native Pigs (KNP). Sequencing analysis identified 10 genetic variants positioned at nucleotides 108 (T>C), 251 (G>A), 324 (C>A), 460 (T>C), 556 (T>C), 559 (A>G), 598 (G>A), 665 (T>C), 920 (G>A) and 1,115 (G>A). The identified sequences were submitted into GenBank with accession numbers (DQ992502-10 and DQ976363) according to the specified locations of each SNP. Clustering analysis revealed that KNP was formed to a major group, showing close genetic relationships with SLA-6*0105, SLA-6*01w01 and SLA-6*w02sa01 alleles except DQ992503. KNP showed the low nucleotide diversity with significant differences of the ratio of ti/tv (transition/transversion) compared with other breeds. The identified variants of the SLA-6 gene are useful information to differentiate phylogenetic relationships between KNP and other pig breeds. The unique results of the SLA-6 SNPs of KNP will serve as reference study for further analyses of gene fixations in evolution studies.

Keywords: SLA-6, Genetic Relationship, Korean Native Pig, Polymorphism, SNP

1. INTRODUCTION

The Swine Leukocyte Antigen (SLA), which is expressed on the surface of most nucleated cells and recognized by CD8+ cells, has been issued in transplantation studies with the precise SLA genotypes and alleles (Ando *et al*., 2003; Barbosa *et al*., 2004; Chardon *et al*., 2001). The MHC region containing huge mutation sites is able to characterize individual differences using several allelic variations due to more than 1500 alleles in human (Giovambattista *et al*., 2001) while limited numbers of allelic variations have been characterized in swine (Crew *et al*., 2004; Ehrlich *et al*., 1987). The genetic information of SLA has been actively studied to understand immune systems and to identify the influence of the SLA genes on immune responses, organ transplantation and disease resistances (Kim *et al*., 2005). According to the question in the human medical issues for understanding mechanisms of immune responses, lots of efforts to solve the questions regarding difficulties in transplantation have been applied with porcine tissues that were believed to be candidate organ materials. Meanwhile, as a candidate experimental animal, the transplantation research seeks availability of Korean Native Pigs (KNP) that appear relatively small body sizes comparing with other breeds even though very limited genetic information for the characterization of KNP is available (Kim *et al*., 2006; Chung and Yoon, 2008; Chung *et al*., 2011). Therefore, it is necessary to characterize genetic variations in the SLA-6 genes for KNP that has been maintained as a closed population for a long-period time.

SLA-6 is in the SLA non-classical region located in pig chromosome 7 (Mallard *et al*., 1989). Even though exact functions of the non-classical SLA genes (SLA-6,

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-7 and -8) have not been determined, it is similarly predicted to genes in the classical SLA I region. As glycoproteins with the potential of binding peptides, a study (Martens *et al*., 2003) reported that swine showed negative homologous to the SLA-6 gene for human and mice while expression of the SLA-6 mRNA transcripts in a variety of tissues presented similar patterns with HLA-E (Park *et al*., 2010). A previous study reported that SLA-6 comprising 270 amino acid residues expressed as monomorphic proteins (Chardon *et al*., 2001). In addition, it is really important to confirm expression patterns according to the genetic variants and to verify polymorphisms in KNP that has never been analyzed regarding SLA-6. Collecting genetic variants in coding regions of SLA-6 should be an initial step to characterize variability and identify the SLA-6 alleles for KNP. Thus, this study was aimed to verify SLA-6 and provide genetic and phylogenetic information of KNP for further studies.

2. MATERIALS AND METHODS

2.1. Experimental Animals

This analysis was designed to verify genetic variants of the coding regions of SLA-6 and to find genetic differences with previously defined Korean pigs and other pig breeds. KNP has been maintained to preserve genetic materials at National Institute of Animal Science (NIAS) in Korea and a total of 30 KNP aged 30 weeks from different sired lines were randomly selected. The pedigree analysis confirmed no significant genetic relationships, showing less than 0.01 inbreeding coefficients. The animals registered in the national database had standard breeding programs under the restricted guidelines provided by NIAS and the animals were fed up with standardized diets with the NRC (2000). After Slaughter at the facility of NIAS, the harvested spleen tissues (10g) were cut into small sections (2-3 mm cubes) and placed into a -70°C deep freezer until uses.

A total of 28 Yorkshire pigs, which had no particular genetic relationships according to the pedigree from NIAS, were used for the genotyping of microsatellite loci to compare genetic variability against KNP. The pedigree analysis ascertained that Yorkshire pigs having less than 0.01 of inbreeding coefficients were selected from sire lines at NIAS. Approximately, 5 mL of blood samples was taken from the jugular vein after post-weaning tests of 150 days. This experiment involving procedures and animals was approved by the ethics and welfare committee of NIAS.

2.2. Analysis of Microsatellite Loci

Even though the pedigree analysis described no significant genetic relationships among 30 KNP animals that were offspring of frequently used sire lines, small numbers of animals open give biased results because of extremely low genetic variability. Therefore, a total of 19 microsatellite loci were used to verify whether the selected population of KNP can have enough genetic variability or not. The used 18 Micro Satellite loci (MS), which were distributed in all over the pig Chromosomes (Ch), were presented in **Table 1**. For the optimal PCR conditions, a gradient PCR was performed with 2 uL of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 25 mM of dNTP, 10 pmol of each primer, 50 ng of genomic DNA and 2 units of Taq DNA polymerase (Gibco BRL, USA) in a final volume of 20 uL (MJ Research, PT-200, Watertown, MA). The genotyping was conducted using an ABI3730XL Genetic Analyzer (Applied Biosystems, USA).

2.3. Primers for Polymerase Chain Reaction

Primer selections for SLA-6, which consisted of 7 exons in 1,087 bp length, were based on the swine coding sequence (a GenBank accession no. AF464007) with options of 60% GC content and 1,400 bp of length. The forward and reverse primers were AAACT CAGAT CCGCC ACAGA (at nucleotide positions 13-32) and TTCAC ACAGC TTTAT TGAAG CAA (at nucleotide positions 1,385-1,407), respectively. An internal forward primer positioned at 431 to 450 (ATTGG CAACC ACAAC CATAG) was also used to confirm correct amplification products because high sequence similarities between genes around the SLA classical and non-classical regions.

2.3.1. cDNA Synthesis

Homogenization was performed with 2 g of tissue samples in 1 ml of Trizol (Sigma, USA) at liquid nitrogen. The total RNA was extracted from 30 KNP pigs by using a kit (RNeasy Midi kit, Qiagen) followed by manufacturer's guidelines and the total RNA was in 50 uL of RNase-free water. The first strand was synthesized by oligo dt primers with 5 X First-Strand Buffer, 0.1M DTT, RNasin and SuperScript™ III Reverse Transcriptase (Invitrogen) standing for 5 min at 25°C, 2 h at 50°C and 15 min at 70°C.

Table 1. Number of alleles, expected heterozygosity and gene diversity for the microsatellite loci in pig populations

KNP (30)								Yorkshire (28)							
Loci	A[1]	He[2]	GD[3]	Loci	A	He	GD	Loci	A	He	GD	Loci	A	He	GD
S0002	2	0.443	0.444	S0227	2.0	0.501	0.503	S0002	3	0.574	0.567	S0227	4.0	0.587	0.588
S0005	5	0.559	0.558	S0228	7.0	0.847	0.850	S0005	7	0.833	0.834	S0228	6.0	0.773	0.702
S0026	6	0.793	0.793	S0386	5.0	0.726	0.729	S0026	2	0.503	0.500	S0386	4.0	0.678	0.688
S0068	3	0.503	0.502	S0355	5.0	0.730	0.733	S0068	2	0.439	0.438	S0355	7.0	0.788	0.786
S0090	4	0.703	0.707	SW24	4.0	0.627	0.639	S0090	3	0.568	0.563	SW24	3.0	0.472	0.539
S0101	3	0.384	0.384	SW787	4.0	0.595	0.596	S0101	2	0.225	0.229	SW787	4.0	0.439	0.438
S0155	5	0.397	0.399	SW857	4.0	0.703	0.706	S0155	2	0.284	0.283	SW857	6.0	0.757	0.756
S0218	4	0.636	0.643	SW911	4.0	0.650	0.649	S0218	3	0.457	0.462	SW911	4.0	0.562	0.563
S0225	2	0.089	0.089	SW951	3.0	0.513	0.508	S0225	2	0.254	0.255	SW951	2.0	0.503	0.502
S0226	2	0.406	0.405	Mean	3.9	0.491	0.570	S0226	3	0.551	0.550	Mean	3.6	0.469	0.543

[1]Number of allele; [2]Expected Heterozygosity; [3]Gene diversity per locus parentheses are numbers of individuals genotyped in this analysis

To amplify cDNA segments, 2 uL of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCL, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 10 mM dNTP, 10 pM of each primer, 30 ng of cDNA and 2 units of Taq DNA polymerase (Gibco BRL, NY) in a final volume of 20 uL were used. After denaturation at 95°C for 3 min, a total of 35 cycles were adapted for denaturation at 94°C/1 min, annealing at 61°C/1 min and polymerization at 72°C/2 min.

2.4. Sequencing Analysis

Amplified PCR fragments of SLA-6 showing approximately 1,395 bp were identified and purified from 1.2% agarose gels using the Nucleotrap gel purification system (Clontech, CA). Each fragment from individuals was inserted into a pGEM T-easy vector at 4°C for overnight and transformed into the DH10B competent cells (Promega, WI). The plasmid was purified using a Mini-plasmid prep kit (Qiagen, CA) and sequencing was conducted with an ABI3730XL Genetic Analyzer using T7 and SP6 universal primers. PCR and sequencing reactions were duplicated to minimize base-calling errors. A total of 460 clones from 30 KNPs were sequenced to determine genetic variants in the coding regions.

2.5. Statistical Analysis

The GENEPOP (Version 3.3) package calculated the number of alleles, allele frequencies, expected heterozygosity and gene diversity. Individual sequences of cDNA were aligned by the Seqman program and clustered by the Megalign program of DNAstar version 6.1. The identified sequences containing SNP were submitted into GenBank with accession numbers and compared with previously submitted SLA-6 alleles from the Immuno Polymorphism Database (IPD). A phylogenetic tree was constructed with a DNAdist option (MEGA version 5.2) for identifying genetic distances between the SLA-6 alleles. A total of 16 unique alleles of the SLA-6 gene from GenBank with accession numbers were used to characterize phylogenetic relationships among alleles as well as breeds including KNP. Statistical summaries for nucleotide diversity, substitutions and frequencies were described using ARLEQUIN version 2.0.

3. RESULTS

3.1. Analysis of Repeat Units

The genetic variability was tested with 19 microsatellite loci for 58 individuals from KNP and Yorkshire pigs and numbers of alleles, expected heterozygosity and genetic diversity were estimated (**Table 1**). The highest number of alleles was 7 in MS markers (S0228, S0005 and S0355) and the lowest number of alleles was 2 in several markers. Overall average number of alleles for KNP was higher (3.9) than Yorkshire (3.6), caused by either high genetic variability of KNP than Yorkshire or different numbers of individuals that had significant different genetic backgrounds. The same patterns of the high average gene diversity and heterozygosity of KNP comparing with Yorkshire were observed. The analysis estimated the average gene diversity of KNP (0.492) that was lower than Yorkshire (0.648), but the results were similar to the report (0.489) of natural populations (Rahman *et al*., 2006).

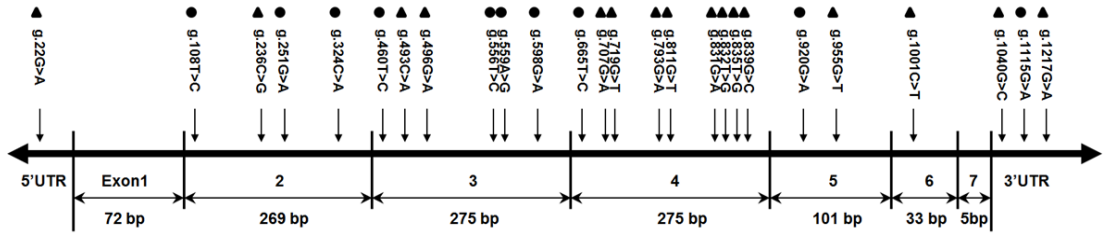

Fig. 1. A map has been constructed with the identified SNP for the SLA-6 gene showing nucleotide substitutions. Exons and UTRs were presented with the length of sequences. The genetic variations in the SLA-6 gene were confirmed using 16 previously defined SLA-6 alleles (triangle) as well as 10 identified sequences for KNP (circle) in this study. The nucleotide positions were based on a GenBank accession number (DQ992510) that was generated from this study.

Table 2. Genetic variations of the SLA-6 gene in KNP

SNP[1]	Substitution		Variability[2]	Minor allele frequency	Coding region	GenBank accession Number[3]
	Nucleotide	Amino acids				
g.108T>C	T>C	L-P	0.047	0.008	2	DQ992505
g.251G>A	G>A	G-S	0.061	0.011	2	DQ992503
g.324C>A	C>A	P-Q	0.433	0.156	2	DQ992506
g.460T>C	T>C	-	0.084	0.016	3	DQ992504
g.556T>C	T>C	-	0.105	0.022	3	DQ992509
g.559A>G	A>G	-	0.061	0.011	3	DQ992502
g.598G>A	G>A	-	0.792	0.135	3	DQ976363
g.665T>C	T>C	C-R	0.072	0.014	4	DQ992507
g.920G>A	G>A	V-M	0.061	0.011	5	DQ992503
g.1115A>G	A>G	-	0.034	0.005	3’	UTRDQ992510

[1]The locations were based on DQ992510; [2]The variability was estimated by the entropy options that measure amount of variation in a column in an alignment; [3]The identified sequences according to the genetic variants were submitted into GenBank with accession numbers

The MS markers have been used to ensure that the KNP population is not much inbreed and isolated against standardized pig populations. If the result of MS analysis shows significantly low genetic variability in KNP, the selected animals may not be a representative for KNP. From the results, a critical opinion that genetic variability of KNP should be greater than Yorkshire meets the expected criteria that the selected individuals of KNP have enough genetic variability to search Single Nucleotide Polymorphisms (SNP) of SLA-6.

3.2. cDNA Analysis

Electrophoretic separations detected sizes of the isolated cDNA as the full-length SLA-6 genes varying 950 to 1430 bp. Due to the variation of sequence lengths, amplified products less than 1,000 bp, which occupied approximately 5% in this analysis, were compared with previously deposited sequences in GenBank. After verification of sequences, the fragment was assumed for containing missing parts in coding regions and therefore, the sequence length less than 1,000 bp was eliminated for further analyses. Successfully amplified cDNA of SLA-6 was confirmed as 1,395 bp that contained the whole coding regions with 5’ and 3’ UTRs. An internal primer was used to verify SLA-6 because genes in the SLA regions showed high sequence similarities that may interrupt amplifications. The sequence alignments verified that coding regions were started from a nucleotide position 29 and ended at 1,138 based on sequences from a GenBank accession number (DQ992510) generated from KNP in this study. Nucleotide sequences in coding regions for all KNP were compared to identify genetic variants. As shown in **Fig. 1**, a total of 26 SNP, which contained previously identified 16 SNP according to the characterization of the SLA-6 alleles from GenBank, have been identified and the newly detected 10 SNP in this study using KNP were presented with a shape of circles. The identified sequences were submitted into GenBank with accession numbers according to the SNP positions (**Table 2**).

The present findings are in a good agreement with the report that a stop codon in exon number 7 was observed and resulted in total 270 amino acids (Charon, 2001). The sequence analysis observed amino acid substitutions in DQ992503 (G>S and V>M), DQ992505 (L>P), DQ992506 and DQ992508 (P>Q) and DQ992507 (C>R). Overall, exon numbers 2 and 3 occupied more than 70% of genetic variants. The analysis confirmed 4

SNP (nucleotide positions 22, 1,040, 1,115 and 1,217) located in both 5' and 3' UTR regions. The sequence comparisons identified major differences between KNP and SLA-6*0105 (Meishan), showing variants at nucleotide positions g.936T>G and g.1096G>A that were observed in Meishan only. Minor Allele Frequencies (MAF) were calculated and g.324C>A was revealed the highest allele frequency (0.156). Even though, MAF of g.598G>A was lower than g.324C>A, the variability of g.598G>A revealed the highest value (0.792) compared with g.324C>A (0.433). Other than these 2 SNPs, the rest of SNPs showed extremely low MAF values.

3.3. Genetic Diversity

To investigate genetic distances between KNP and 16 representative SLA-6 alleles (**Table 3**), a phylogenetic tree was constructed (**Fig. 2**). A close genetic distance between the identified KNP in this study and previously defined 3 alleles (SLA-6*0101, SLA-6*0102 and SLA-6*w04jh01) using Korean pigs has been hypothetically expected due to the same native breed. However, the most obvious point was that the identified sequences of KNP showed differences in a varying degree from the previously identified SLA-6*0101 allele (DQ883215) that presented significant genetic similarities with Large White (AY463540). In addition, all KNP individuals were clustered into a major group except DQ992503 that was slightly departed from KNP and the results were caused by SNP located at nucleotide positions g.324C>A and g.598G>A. Significantly high sequence similarities have been observed among pig populations such as KNP, Meishan (SLA-6*0105), NIH (SLA-6*0102 and SLA-6*w02sa01) and western (SLA-6*01w01) breeds. In contrast, other SLA-6 alleles (SLA-6*0104 and SLA-6*0103) of the Meishan breed did not show close genetic distances with KNP.

The low overall polymorphic information (2.311) and nucleotide diversities (0.002) were estimated in KNP against Meishan, previously identified Korean pigs (KOR) and OUT consisted of Yucatan, NIH and LW (**Table 4**). The results may characterize specificities of genetic constitutions of KNP that show a little degree of the inbreeding status. For the convenience of further evidence, the nucleotide substitutions by transitions and transversions were revealed that KNP had the highest ti and the lowest tv, resulting in a significantly high ti/tv ratio (9.000) compared with others (average 0.578). The results imply that KNP may be a little away from the major evolutionary pathways comparing with other pig populations.

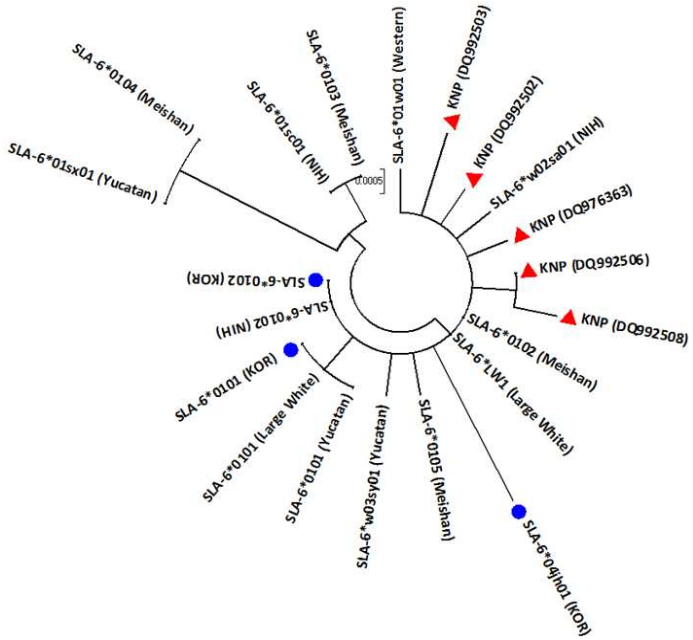

Fig. 2. The phylogenetic relationships of the SLA-6 sequences among Korean Native Pigs (KNP) and 16 SLA-6 segments (complete cDNA sequences from GenBank representing the SLA-6 alleles). The phylogenetic tree was constructed with DNADist in Bioedit program version 7.0.1. Accession numbers (DQ992502-DQ992510 and DQ976363) represented KNP (triangles with red color) in this study and KOR represented previously defined Korean pigs (circles with blue color).

Table 3. The SLA-6 alleles, breeds and accession numbers that used to determine genetic distances using SNP

Group	Allele	Previous designation	Breed	Accession number	Reference
SLA-6*01	0101	z	Yucatan	AF464006	Martens *et al*., 2003
			Large White	AY463540	Crew *et al*., 2004
	0101		KNP	DQ883215	GenBank
	0102		NIH	AF464020	Martens *et al*., 2003
		LW1	Large White	AY247772	GenBank
	0102		Meishan	EU432054	GenBank
	0102	a	KNP	DQ883216	GenBank
	0103		Meishan	EU432055	GenBank
	0104		Meishan	EU432056	GenBank
	0105		Meishan	EU432057	GenBank
	W04jh01		KNP	DQ883214	GenBank
	01we01	We1	Western	AY247771	GenBank
	01sc01	c	NIH	AY459304	Martens *et al*., 2003
	01sx01	x	Yucatan	AF464008	Martens *et al*., 2003
SLA-6*w02	W02sa01	a	NIH	AF464019	Martens *et al*., 2003
SLA-6*w03	W03sy01	y	Yucatan	AF464007	Martens *et al*., 2003

Table 4. Number of polymorphic sites, nucleotide diversity, transition/transversion ratio and compositions of nucleotides

		Polymorphic region			Count		
Breed[1]	N	Polymorphic information	Nucleotide diversity	ti/tv	ti[2]	tv[3]	SNP
OUT	9.00	2.556	0.0023±0.001	0.667	4.00	6.00	10
KOR	3.00	3.333	0.0029±0.001	0.667	2.00	3.00	5
KNP	30.00	2.311	0.0021±0.001	9.000	9.00	1.00	10
MES	4.00	3.607	0.0033±0.001	0.400	2.00	5.00	7
Mean	11.50	2.952	0.0026±0.001	1.133	4.25	3.75	8

[1]OUT: All breeds except Korean Native Pig (KNP) and Meishan (MES) breeds; KOR: Previously defined Korean pigs; [2]ti: Transition (T/C and A/G); [3]Tv: Transversion (A/T, T/G, C/G and A/C)

4. DISCUSSION

4.1. Microsatellite Loci

As a study (Takezaki and Nei, 1996) determined that the average heterozygosity was ranged from 0.5 to 0.8 in natural populations to be useful markers for measuring genetic variations, the present study marginally tended to agree the ranges even though the value was slightly low (0.491). If there was a high selection pressure in KNP for a long-period time to produce well-known favorite features of native pigs, low levels of heterozygosity and gene diversity would be expected. Even though ranges of the expected heterozygosity in KNP (0.491) were greater than that of Yorkshire (0.469), the result may not be directly referred to a natural population for KNP. In fact, this is somewhat expected because KNP has not been actually selected for commercial purposes due to no popularity in markets. In addition, the average weight at days 150 was significantly lower than any other pig breeds and therefore, traits of average daily gain and feed efficiency were not compatible against the major pig breeds such as Yorkshire, Landrace, Duroc and so on. Therefore, it is technically difficult to describe KNP as a natural population that means inbreeding coefficients should be higher than expected.

As the hypothetical idea assumed that KNP was in a significantly high inbreeding status due to managements of populations with limited numbers of sires that were maintained for preservation purposes, the low genetic diversity of KNP was eventually expected. In contrast, the results showing high genetic diversity compared with Yorkshire implied that significantly biased mating systems have not been adapted in KNP with small proportions of sires. The results may also assume that the genetic variability of KNP is placed in a marginal and natural breeding stage. The result of heterozygosity was also agreed with other studies that have used native pig breeds showing ranges of heterozygosity from 0.49 to 0.70 (Rothschild *et al*., 1984; Shigenari *et al*., 2004; Smith *et al*., 2004; Ednaldo *et al*., 2012). Consequently,

the gene diversity of KNP is not significantly departed from Yorkshire and therefore, the selected KNP is to be an appropriate population as a representative breed regarding searching SNPs in SLA-6.

4.2. Sequence Analysis

Genetic variants of SLA-6 derived from spleen tissues of KNP may be unique characteristics and therefore, the SNP can be used to develop an inbreed line and to differentiate pig breeds. Even though there were not enough reports for SNP due to limited polymorphisms in SLA-6, a study (Smith *et al*., 2004) found 2 SNPs at nucleotide positions g.465G>A (AF464019) and g.679G>A (AF464007), but these SNPs were not detected in this analysis. Alignments identifying SNPs between KNP and other SLA-6 alleles detected high sequence similarities, showing only differences at 10 nucleotide positions (108, 251, 324, 460, 556, 559, 598, 665, 920 and 1115) that may account for the major differentiation of the SLA-6 alleles. In addition, the identified SNPs, which were not shown in the previously determined SLA-6 alleles from GenBank, may aid to characterize breed specificities as well as allelic types of individuals. An earlier study mentioned that SLA-6 has a stop codon in exon 7 leading to a matured SLA-6 protein comprising only 270 Amino Acid (AA) residues rather than 274 AA (Chardon *et al*., 2001) and the present study confirmed 270 AA.

The ratio of ti/tv, which is a parameter for phylogenetic estimations, intended to reflect the fact that nucleotide substitutions are not all equally likely between pig breeds. The substitution presented nucleotide diversities according to the transitions (T>C and A>G) and transversions (A>T, T>G, C>G and A>C) while performing a comparative analysis of sequences between populations. In general, a ratio of ti>tv ranges from 1 to 2 because transversions (A>C and T>G) are 3 times less frequency in the animal genome (Keller *et al*., 2007). The present study compromises similar results except KNP that showed extremely high frequencies of transitions that leads the high ratio of ti/tv (an average 9.0). The results are caused by more substitutions of T/G and A/C while low transversions of A/T and C/G are presented. Therefore, the results imply that KNP holds significantly different aspects of genetic diversities in terms of general patterns for genetic segregations of genes from the evolutional pathways. In addition, the mutation sites in this analysis are unique positions that can identify breed specificity.

In fact, SNPs are significantly used to determine the SLA haplotypes that can characterize population resources when trying to identify indigenous animal populations. Finding new SNPs in the SLA region, therefore, may give us valuable information to decide haplotypes, as well as to present distinguishable diversities of animal populations. The newly identified SNPs in this study can provide useful genetic information to phylogenetic studies and the SNPs in KNP would have maximal relevance to explain pig diversities as well as to determine genetic characteristics of individual animals.

4.3. Phylogenetic Analysis

It is assumed that population sizes of KNP were extremely small over the past decades due to a shortage of commercial popularity causing dramatic decreases numbers of animals and allele frequencies. A limited portion of animals was selected and distributed either naturally or artificially and therefore, the low allelic distribution showing low heterozygosity would be expected. However, the analysis revealed a relatively high heterozygosity when KNP has been compulsively assigned to SLA-6*w11jh01 and SLA-6*w11jh02 alleles, showing approximately 57% of heterozygosity that was greater than that of reports (51%) using the SLA-DQ1B gene (Takezaki and Nei, 1996). The heterozygosity of KNP is still low, comparing with naturally managed populations; for example, the heterozygosity of the BoLA-DRB3 gene has been reported as 47 to 93% using indigenous animals (Yang *et al*., 2003; Zhang *et al*., 2003). It is, therefore, kindly expected that allele frequencies of SLA-6 for KNP are slightly skewed and fixed in a favorite way of selections. In fact, most domesticated pig breeds tend to be genetically uniformed as a result of high levels of gene flow among populations and artificial selections with highly reproductive individuals. As a matter of fact, KNP has been preserved for a long-period time without introducing out breeds and this is a critical point to maintain KNP as a pure line. In addition, it is a beneficial aspect for using closed populations with significantly high inbreeding coefficients to make an experimental population with reducing potential variations and increasing gene fixations. Most domestic animals are also highly selected for economic important traits such as average daily gain or meat production and the directional selection may reduce genetic diversity as well as genetic variability. The high levels of artificial selection through the intensive uses of elite sires can assist increases of reproduction rates with reducing the

effective population size. However, this is not the critical point to explain differences of genetic diversity with limited genetic information for the SLA-6 genotypes and alleles because KNP has not been intensively selected. There is no significant evidence that KNP has been highly inbred based on several values of genetic variability. Furthermore, if heterozygous markers of SLA-6 in KNP are lower than other domestic populations, the selected individuals with the SLA genotypes and alleles may be helpful to maintain pig populations for specific purposes in animal breeding areas. The low allelic diversity of the SLA genes can be used for management of KNP in order to increase population fitness. However, a current situation of genetic variability of KNP is too wide to use as a highly standardized population and therefore, KNP should be maintained to reduce and standardize genetic variability for further uses in model animals.

5. CONCLUSION

According to the identified genetic information for sequences and alleles of the SLA-6 gene, comparisons and phylogenetic analyses confirmed that KNPs in this analysis were significantly differed from the previously reported Korean pigs (SLA-6*0101, SLA-6*0102 and SLA-6*04jh01) and other pig breeds. Two genetic variants (g.324C>A and g.598G>A) out of 10 should be informative according to the MAF that were 0.156 and 0.135, respectively. Therefore, genetic variants of the SLA-6 gene in the present report provide candidate genetic markers for further analysis regarding management of KNP in order to fix SLA genotypes and differentiation of pig breeds. Consequently, this study characterizes KNPs using the SLA-6 gene and alleles and the variation of nucleotide sequences is also valuable for developing an SLA genotyping protocol. The results of SLA-6 SNPs of KNP reported here will serve as the reference study for KNP that can be used for the animal model based on requirements of gene fixation.

6. ACKNOWLEDGEMENT

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7. REFERENCES

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